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Influence of Alcoholic Extract of Horse Kidney on Eimeria tenella Infection in Chicks*

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"Forssman hapten" is a term applied to a complex antigenic substance which, when injected into rabbits, elicits the production of a serum antibody that is capable of hemolyzing sheep erythrocytes in the presence of complement. The organs of the guinea pig served as the source of the antigen in Forssman's original experiments, but it is known to occur in a considerable number of other living organisms. The kidney of the horse is a particularly good source of true forssman hapten (Brunius, 1936). It has been shown that alcoholic extract of horse kidney, made according to the method which Brunius found satisfactory for obtaining the source material of the Forssman hapten, is capable of potentiating agglutination of duck erythrocytes by chicken plasma (Becker and Schwink, 1953). The destruction of duck erythrocytes labeled with *Plasmodium lophurae* in the blood stream of chicks, however, was not significantly accelerated by intravenous injections of the same horse kidney extract. Though the reason for the negative outcome was not known, one possibility was considered to be stronger affinity of the fixed tissues for the extract than of the blood plasma. Since the coccidium *Eimeria tenella* undergoes the greater part of its asexual and sexual development in the mucosa of the caeca of chickens, tests were made of the effect of injections of horse kidney extract on the development of this parasite.

Materials and Methods

The procedure for preparing the extract was the same as the one for duck-liver extract (Becker, Schwink and Probst, 1952) and the horse-kidney extract previously used (Becker and Schwink, 1953). It involved grinding the fresh horse kidneys, extraction with acetone, drying, extraction with ethyl alcohol, acetone precipitation, drying the precipitate and redissolving it in physiological salt solution. One cc. of the solution was equivalent to 1.0 g. of the fresh tissue. To each chick of the various test series was administered 1.0 cc. of the solution at the time of inoculation with oocysts and on each of the following six days in Series A, B and C, and of the following five days in Series D and E.

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The strain of *Eimeria tenella* employed was started with oocysts in scrapings from a thoroughly washed piece of caecal mucosa from a field case, and had been passed through chicks at intervals in order to maintain the infective potency of the oocysts. In order to insure against both loss of chicks from hemorrhage and plugging of the caeca of survivors, a single infective dose of 500 recently sporulated oocysts was injected directly into the crop through a rubber catheter attached to a Luer syringe. The challenging dose used in Series E consisted of 1500 sporulated oocysts on the ninth day of the primary infection. The chicks were Hampshire Reds varying in age from 19 to 39 days. They were fed balanced starting and growing mashes of known formula that had not been supplemented with vitamin B_{12}, antibiotics, or coccidicidal or coccidistatic drugs.

The chickens were kept in cages with bottoms of wire mesh over flat collecting pens. The faeces were thoroughly disintegrated, screened and mixed with water, and oocyst counts were made by the dilution method using a haemocytometer. Collections were made on the seventh day and through the eleventh day. The chickens were killed on the eleventh day and examined for plugged caeca. None of the latter were seen in chickens inoculated with 500 oocysts. The caeca were cut into small pieces, homogenized with a Waring blender, and the number of oocysts in the suspension was added to the total in the feces. The oocyst counts appearing in the tables are total counts for the collection period. In the case of Series E, counting was carried through the twentieth day.

Tests for significance were made as previously described (Becker, Brodine, Marousek and Byrd, 1949).

Results

The results expressed in mean oocyst counts with standard deviations appear in Table 1. The mean total oocyst counts for the controls were higher than those for the tests in Series A, B, C, D, and E. The probabilities in Series A, B, C and D were approximately 0.05, 0.05, 0.35 and 0.05, respectively. In these series individual counts were made. In Series E there were several birds in each cage, so that only an average of the total counts was obtained. The results as a whole indicate that the injections of horse kidney extract retarded the production of oocysts in the recipients.

The coefficients of variation were computed as a measure of the amount of variability within the groups. It is notable that in each series the coefficient of variation for the test group corresponded fairly closely with that for the control group. The second notable feature is the wide difference in variability among the

Table 1

Oocysts (in millions) produced during infection with *Eimeria tenella* in chicks treated with physiological salt solutoin (controls) and with horse kidney extract (tests).

Series	Groups	No. of Chicks	No. Oocysts M and SD	Range in Groups	Coefficient of Variation	Mean No. Oocysts in Reinfection
	Tests	5	11.3 ± 8.9	3.6—27.3	78.8%	
A	Controls	5	67.8 ± 41.1	13.6—133.8	60.6%	
	Tests	5	32.3 ± 13.7	20.3—57.9	42.4%	
B	Controls	5	73.0 ± 29.4	31.2—102.4	40.3%	
	Tests	12	17.6 ± 20.9	2.7—39.6	113.1%	
C	Controls	12	27.1 ± 30.1	4.4—118.0	111.1%	
	Tests	5	22.5 ± 10.9	9.9—34.7	48.4%	
D	Controls	6	52.0 ± 25.8	25.5—86.0	49.6%	
	Tests	13	55.9			94.2
E	Controls	14	85.8			50.8

series ranging from 42.4 and 40.3% in Series B to 113.1 and 111.1% in Series C. When the yields of oocysts from comparable dosages of oocysts are taken into consideration along with the coefficients of variation, it appears that the culture with the lowest vitality, the one used in Series C, produced the greatest variability in the number of oocysts produced in each bird.

Series E was employed as a sort of final confirmation of the results obtained in the previous series. It occurred to the authors at this late hour to reinfect the chicks of each group to test for the net resistance of the two groups after recovery. Accordingly, each bird in the series was reinfected with 1500 sporulated oocysts. Horse kidney extract was not administered during this test. It had been anticipated that the test group would have acquired the greater net resistance as a result of both the protective action of the horse kidney extract and the infection. To our intense surprise the reverse was the case, for the test series eliminated an average of 94.2 million oocysts per bird and the control series 50.8 million.

To determine whether the horse kidney extract afforded clinical protection against the coccidium, six 39-day-old test chicks were injected with horse kidney extract and six controls with physiological salt solution. Each chick was infected with 30,000 sporulated oocysts. Three chicks of the test group and two of the control group were dead by the sixth day. Autopsy showed severe hemorrhage of the caeca. The surviving nine chicks were killed and examined on the sixth day. All showed extensive cecal hemorrhage, with little or no difference in severity between the two groups.

Discussion

So far as the authors can determine, no coccidial infection has ever been influenced experimentally by what might be called immunological methods, except through active immunization with the living organism (cf. Becker, 1935; Reyer, 1941). The horse kidney extract used in the present experiment has been shown to potentiate hemagglutination of duck erythrocytes by chicken plasma (Becker and Schwink, 1953). It has now been shown that, when injected into chicks, it effects a moderate though significant reduction in the number of oocysts eliminated by the host. It cannot, however, be said definitively in this case that the reduction in oocysts eliminated is due to either indirect or direct potentiation of an antibody. The fact that reinfection of one series, after the administration of the extract was discontinued, resulted in higher oocyst yields in the test group than in the control might be taken as evidence that the results to the contrary obtained in the primary infection were due to passive potentiation of an innate tissue antibody. One argument against the validity of such evidence is the fact that no clinical protection was conferred by the administration of the horse kidney extract in the one series attempted. This experiment, however, might have resulted in showing some degree of clinical protection had the infective dosage been lighter.

It should be stated here that one extract was made of the livers of actively immunized (through repeated previous infection) chickens by the same method, and another of the combined caeca, kidneys and spleens. These were tested in chickens for their effect on oocyst yields by the same procedure as with the horse kidney extract. Six chicken-liver-extract recipients eliminated a mean of 74.0 million oocysts, while the six controls eliminated 74.9 million oocysts. Six chick recipients of the extract of the other organs eliminated a mean of 59.9 million oocysts, while their six controls eliminated a mean of 51.0 million oocysts. These negative results indicate the absence of the active factor in the tissues of the immunized chicken.

Summary

An alcoholic extract of the kidney of the horse was made by Brunius' method for obtaining the source material of the Forssman hapten. It has been shown to possess the property of potentiating the agglutination of duck erythrocytes by most chicken plasmas, but when injected intravenously did not confer protection on chicks inoculated with duck erythrocytes infected with *Plasmodium lophurae*. In the present work, however, it is shown that this horse

kidney extract, when injected intravenously into chicks inoculated with *Eimeria tenella,* brings about a moderate reduction in the yields of oocysts. If the action of the extract in the living bird parallels its action in potentiating hemagglutination in the test tube, its effect could be ascribed to neutralizing an innate inhibitor of an innate tissue antibody. Conditions in the host, however, are so complex that this explanation is offered only as a possibility.

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